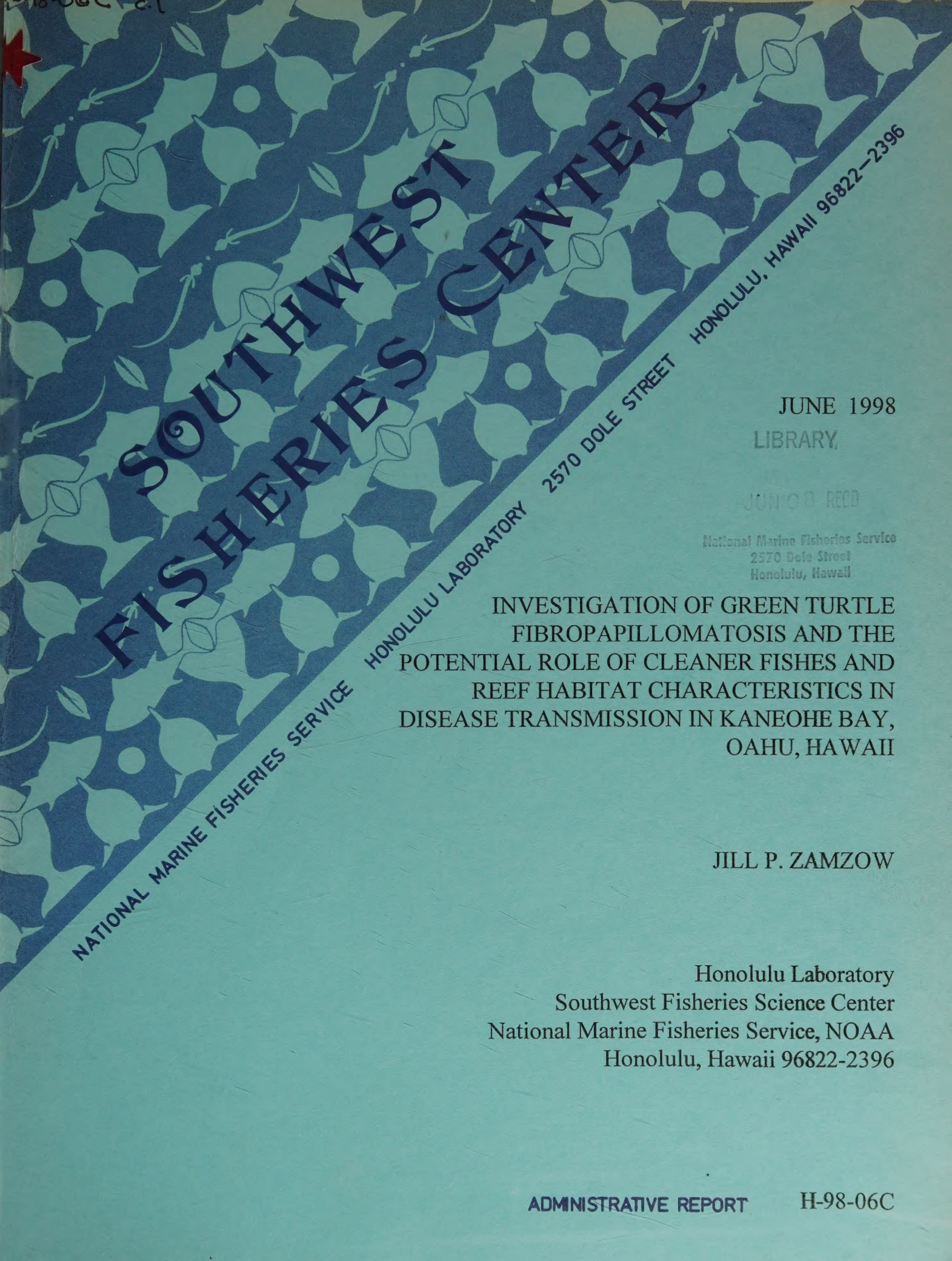




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INVESTIGATION OF GREEN TURTLE FIBRO-PAPILLOMATOSIS AND THE POTENTIAL ROLE OF CLEANER FISHES AND REEF HABITAT CHARACTERISTICS IN DISEASE TRANSMISSION IN KANEOHE BAY, OAHU, HAWAII

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INVESTIGATION OF GREEN TURTLE FIBROPAPILLOMATOSIS AND THE
POTENTIAL ROLE OF CLEANER FISHES AND REEF HABITAT CHARACTERISTICS
IN DISEASE TRANSMISSION IN KANEOHE BAY, OAHU, HAWAII

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NOT FOR PUBLICATION

PREFACE

This report provides information on the possible role of cleaner fishes in relation to the tumor-forming disease known as fibropapillomatosis currently afflicting Hawaiian green turtles, *Chelonia mydas*. The research conducted was sponsored in part by a contract from the Southwest Fisheries Science Center Honolulu Laboratory awarded to Dr. George Losey of the University of Hawaii's Department of Zoology and Hawaii Institute of Marine Biology.

The cause of fibropapillomatosis, a debilitating and often fatal disease, remains unknown although a multifactoral viral etiology with environmental cofactors has been implicated. The disease represents a potentially serious threat to the long-term recovery and healthy maintenance of green turtle populations at several locations worldwide, including Hawaii, Australia, Florida, and areas of the Caribbean. In addition, fibropapillomatosis has recently been histologically confirmed in east Pacific populations of the olive ridley, *Lepidochelys olivacea*.

Because this report was prepared by an independent investigator, its statements, findings, conclusions, and recommendations do not necessarily reflect the views of the National Marine Fisheries Service, NOAA, U.S. Department of Commerce.

George H. Balazs
Zoologist
Marine Turtle Research Program
June 1998

This report provides information on the results of a study conducted by the U.S. Department of the Interior, Bureau of Land Management, in cooperation with the U.S. Department of Agriculture, Forest Service, and the U.S. Department of the Navy, Naval Facilities Engineering Command. The study was conducted to determine the effects of the construction of the Hawaii International Airport on the natural resources of the area. The study was conducted by the U.S. Department of the Interior, Bureau of Land Management, in cooperation with the U.S. Department of Agriculture, Forest Service, and the U.S. Department of the Navy, Naval Facilities Engineering Command.

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ABSTRACT

An investigation of the cleaning relationships between Hawaiian reef fishes and green turtles, as well as the resting behavior of the turtles, was conducted. Six 24-hour surveys of cleaning stations and resting sites were performed via time-lapse and real-time video; videotape was later analyzed with event recording software. Twenty-nine individual turtles were identified, eight of them tumored. Fish in Kaneohe Bay, Oahu did not selectively clean tumored areas of the turtle. Carnivorous cleaners in Hanauma Bay, Oahu did target tumors selectively. The cleaning sites in Kaneohe Bay were mapped on a fine scale and global positioning system (GPS) coordinates were taken. A survey was conducted to determine the location of cleaning sites around the island of Oahu.

INTRODUCTION

Fibropapillomatosis is a debilitating and frequently fatal disease of green turtles, *Chelonia mydas*, in the Hawaiian Islands. The disease has reached epidemic proportions in green turtle aggregations occurring in foraging habitats in Hawaii as well as in Florida and at certain other areas worldwide (Balazs and Pooley, 1991). In Hawaii, the disease was first documented in 1958 as a rare occurrence in Kaneohe Bay, Oahu (Balazs, 1991). The prevalence of afflicted turtles at this location has been severe for the past 10 years and continues in the present.

Fibropapillomatosis is characterized by the formation of lobulated fibrous tumors on all skin surfaces, in the eyes, and less frequently, on the internal organs (Herbst and Klein, 1995). In Hawaiian green turtles the disease is unique and particularly damaging to turtles in that tumors also form in the oral cavity causing the obstruction of both breathing and the ingestion of food (Balazs and Pooley, 1991).

The cause of fibropapillomatosis is unknown, but the disease has been conclusively demonstrated, through the use of tumor homogenates in captive turtle studies, to be transmissible and infectious (Herbst et al., 1995). A viral-sized agent appears to be the responsible pathogen. While it is essential that this likely virus be isolated, identified and characterized, it is also of urgent importance that research be concurrently undertaken on the transmission among turtles in the wild. Knowledge of these aspects is essential in order to devise effective management plans to control or contain the disease.

Cleaning symbioses between green turtles and various reef fishes that feed on organisms growing on the turtles' skin, scales, and carapace have been reported in the literature for a number of years (Booth and Peters, 1972; Balazs, 1996). A cleaning symbiosis was recently reported in which the Hawaiian saddleback wrasse, *Thalassoma duperrey*, picks the turtle-specific skin barnacle *Platylepis hexastylus* from the skin of the green turtle (Losey et al., 1994).

The objective of this work was to gain an increased understanding of these cleaning relationships and any correlation they may have with green turtle fibropapillomatosis. Reef fishes may serve as vectors for the disease as they move between turtles, or by causing wounds while cleaning may leave the turtle open for infection. Conversely, the cleaning behavior may serve a beneficial purpose in controlling ectoparasites and/or ameliorating tumor tissue. Increased information on these cleaning interactions between fishes and turtles is needed to

determine if a significant impact exists, positively or negatively, with regard to the fibropapillomatosis.

METHODS

This study was conducted over a 5-month period, from 30 April 1997 to 30 October 1997. Four approaches, taken in order to gather the information necessary to accomplish this work, are as follows:

24-Hour Data

Six 24-hour surveys of turtle cleaning and resting sites were performed in Kaneohe Bay, Oahu, Hawaii. Three surveys (two resting, one cleaning) took place at "Reef 42," and three (two cleaning, one resting) at "Mark's Reef" (see Fig. 1). Both underwater time-lapse video recordings (one survey) and underwater real-time remote-video camera recordings (five surveys) were taken. The time-lapse system was set to 1 second of footage every 1 minute, and was deployed at a resting site and left undisturbed for 12 hours. The remote video system was deployed and continually monitored from the surface during daylight hours (including pre-sunrise and post-sunset hours). A pan and tilt mechanism allowed the researcher to monitor 360 degrees around the camera within the limits of visibility. Video was recorded whenever there was a turtle in view.

Data gained from these surveys included the following: number of individual turtles visiting a site, habitat type where events occurred, number of visits by each turtle, severity of tumor affliction, locations of feeding on the turtles (including tumored vs. non-tumored skin areas by each kind of cleaner), the amount of time each resting site was occupied, and fidelity of individual turtles to specific resting sites.

Real-time video footage was analyzed via behavioral event-recording software (BEAST). For each cleaning encounter, the following events were recorded: (1) individual turtle identity (and whether turtle is tumored), (2) number of cleaning bites by *T. duperrey*, (4) whether the bite(s) hit a tumor, (5) duration of herbivorous cleaning, and (6) species of herbivore(s) present. Turtle identity was ascertained by mapping dorsal and ventral barnacles, tumors (if present), sex (if male), estimation of size (small, medium, or large), and any abnormalities such as amputated flippers or other scarring. For each resting encounter, individual turtles were identified, and duration of resting period was noted.

Mapping of Kaneohe Bay Cleaning Sites

Cleaning stations used during this study were mapped on a finer scale than can be found on nautical charts of the region. For purposes of mapping the Mark's Reef site, the researcher was elevated to a height of approximately 40 ft along the mast of the research vessel *Desperado*, and then proceeded to map the surrounding reef area manually. The map of Reef 42 was adapted from a remote sensing image furnished by Eric Hochberg, School of Ocean and Earth Science Technology, University of Hawaii. GPS coordinates of the cleaning sites were also taken.

Comparative Cleaning Sites

Two cleaning sites outside of Kaneohe Bay were surveyed as a point of comparison to the Kaneohe Bay sites. Cleaning stations at Hanauma Bay, Oahu, and off of the Mauna Lani Hotel, South Kohala Coast, Island of Hawaii were investigated via scuba and hand-held underwater videocamera. Videotape was analyzed to identify cleaner species and type of cleaning.

Survey of Dive Tour Operators

A survey of dive tour operators around Oahu as well as University of Hawaii certified academic research divers was undertaken in order to ascertain the number, approximate locations, and seasonality (if any) of cleaning sites that are currently being encountered during diving activities around Oahu. The survey was conducted via telephone interview and electronic mail communication.

RESULTS

24-Hour Data

One hundred forty-four hours were spent in field observations of turtle resting and cleaning stations. Seventy-two hours (three 24-hour periods) were spent at Mark's Reef, and 72 hours (three 24-hour periods) at Reef 42. A total of 20 hours (120,000 s) of real-time video and 85 s of time-lapse video footage were analyzed. The duration of cleaning interactions totaled 20,436 s, and resting interactions totaled 7074 s. Twenty-nine individual turtles were identified, 24 from cleaning bouts, 3 from resting bouts, and 2 involved in both types of interaction. At least 8 of these animals were tumored. Eighty-two turtle visits were analyzed, 10 from Mark's Reef, and 72 from Reef 42. Three hundred ninety-six bites were recorded for the saddleback wrasse, *T. duperrey*, and 2 for the whitespotted toby,

Canthigaster jactator. In addition, herbivorous fishes (Acanthuridae, Chaetodontidae, juvenile Scaridae) spent a total of 4054 s (19.8% of total observations) grazing on the turtles' shells and skin (see Table 1).

Tumors were bitten by *T. duperrey* 11% of the time (spent on tumored turtles) and the one observed bite by *C. jactator* on a tumored turtle was on a tumor. Herbivorous fishes did not bite the turtles' tumors. Herbivory occurred 20.6% of total cleaning time on tumored turtles, and 19.6% of the total cleaning time spent on clean turtles.

Mapping of Kaneohe Bay Cleaning Sites

GPS technology was used to establish an accurate location of each cleaning site within Kaneohe Bay (Fig. 1). Reef 42 is located at 21°28.593'N, 157°49.451'W, and Mark's Reef is located at 21°27.486'N, 157°47.867'W. In addition, the vicinities of both cleaner stations used in the study were mapped in detail to a radius of approximately 50 m (Figs. 2 and 3).

Comparative Cleaning Sites

A total of 618 s of videotape was analyzed from the Kona Coast, and 1183 s from Hanauma Bay. At the South Kohala coastal site, herbivorous feeding only was observed, by *Acanthurus triostegus*, *Zebrasoma flavescens*, and other *Acanthurus* spp. which were unidentifiable due to limitations of water clarity. At least four individual turtles were present at the cleaning site, and none of them appeared tumored. It should be noted that fibropapillomatosis is virtually nonexistent along the Kona coast (Balazs et al., in press). At Hanauma Bay on Oahu, mainly herbivorous feeding was observed, *Acanthurus* spp. However, near the end of the dive, a severely tumored turtle arrived on the station. Five-hundred three s of videotape were taken of this individual. It did not pose, and herbivores did not approach it, but both *C. jactator* and *T. duperrey* approached and bit the turtle's tumors as soon as it came within approximately 1 m of the reef. The turtle flinched and swatted at the fishes repeatedly, and swam away from them. *C. jactator* and *T. duperrey* followed the turtle, both biting it at tumored areas every time it slowed down. The turtle eventually swam away from the reef and the fishes ceased to pursue it.

Survey of Dive Tour Operators

Seven dive tour operators and 82 University of Hawaii academic research divers were polled in order to ascertain the location and seasonality (if any) of cleaning sites around Oahu. This poll yielded the locations of four cleaning sites in addition to the three known for Kaneohe Bay (Fig. 4). The cleaning sites reported were (from east to west): Hanauma Bay, "Turtle Rock" off Waikiki Beach, "Pinnacles" off Ewa Beach, and "Turtle Rock" off Makaha Beach. Additionally, a resting site was reported at Haleiwa Trench on the north shore. Exact GPS coordinates were not available for these sites.

DISCUSSION AND INTERPRETATION OF THE RESULTS

24-Hour Surveys

An unusually small proportion of tumored turtles were observed during this study (28%). During 3 days of hand-catching turtles in Kaneohe Bay (October 14-16, 1997 with personnel of the National Marine Fisheries Service), the observed tumor rate was 84% (n = 13). Due to the nature of data collection, it is possible that small tumors went unnoticed due to water clarity limitations, or orientation of the turtle to the video camera. However, the author is confident that any turtle with an overall tumor score of TS-2 or more would be noted in the data as "tumored" (NMFS scoring system, TS-1 = mild, TS-2 = moderate, TS-3 = heavy, TS-4 = extreme). The rate of heavily tumored turtles (>TS-2) from the aforementioned 3-day study was 23%, as compared to a heavily tumored turtle rate of 17% found by this study; these are comparable rates.

The unusually low rate of tumor bites by *T. duperrey* (11%) in Kaneohe Bay indicates that tumors are not targeted by the wrasses. Tumors can harbor parasites such as leeches (Balazs, 1991), but the wrasses are ignoring this food source. This may be because the skin barnacles offer better nutrition than these parasites, or because the fishes have learned that turtles swim away if tumors are targeted. By avoiding turtle tumors, more food per cleaning interaction can be gained, and this symbiosis can be termed a true mutualism. *C. jactator*, on the other hand, has not adopted a mutualistic strategy. Both Kaneohe Bay data and Hanauma Bay data indicate that *C. jactator* attacks tumored areas of the turtle, most often resulting in the turtle immediately leaving the vicinity. Most fishes of the genus *Canthigaster* both accumulate tetrodotoxin in their flesh and organs, and also secrete substances making them unpalatable to predators (Hoover, 1993). It is possible that these secreted

substances may enter wounds caused by the toby's bite, thereby deleteriously affecting the turtle.

Examination of the resting data revealed an interesting fact: of the turtles known to be male, the mean resting duration was considerably lower than that of the turtles known to be female. This may be an artifact of small sample size, or may actually show relation to turtle size and not sex necessarily, but bears future investigation. Resting interactions indicated that individual turtles do, indeed, show fidelity to a particular resting site, at least over the course of a few hours.

Comparative Cleaning Sites

It would seem that, in Hanauma Bay, cleaning by carnivorous fishes is not a mutualism. It appears that these fish bite at turtle tumors, ignoring clean turtles entirely, and chase tumored turtles away from the cleaning and resting areas. This may result in negative effects to the turtle's health due to increased parasite load and/or hydrodynamic drag from shell growth. The turtle's health also may be compromised if it is unable to "rest," and is continuously forced to use up energy reserves in avoiding these carnivorous fish species. On the South Kohala Coast, no deleterious interactions were observed, nor was any carnivorous cleaning observed.

Survey of Dive Tour Operators

Turtle cleaning sites were found fairly evenly spaced along the leeward shore of Oahu. This may be due to the fact that this is the most commonly dived shore of the island due to easy accessibility, good visibility, and generally good weather conditions. None of those surveyed could offer information as to whether seasonality of cleaning sites seemed to exist. "They're almost always there" seemed to be the most common answer to the question.

CONCLUSIONS AND RECOMMENDATIONS FOR FUTURE RESEARCH

The role of *Canthigaster jactator* as a possibly deleterious factor to turtle health should be investigated. The duration of resting in relation to the sex of the turtle should be investigated. Cleaning sites around Oahu should be surveyed in further detail. Hanauma Bay and any other sites concerning harassment by fishes should be studied.

A pilot study indicated that there are different kinds of feeding bites employed by *T. duperrey*, and that tumored turtles

more often flinch and/or swat during cleaning bouts than clean turtles (31% of bites vs. 18% of bites, respectively). Turtle response to reef fish cleaning should be more thoroughly investigated.

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Table 1.--Observations of Turtle Cleaning Stations in Kaneohe Bay, Oahu.

Turtle Condition	Turtles observed (N)	<i>T. duperrey</i> bites (N)	Tumor bitten (N)	Herbivory duration (seconds)	Total time observed (seconds)
Tumored	8	147	16	1046	5076
Clean	16	249	0	3008	15360
Total	24	396	16	4054	20436

Table 2.--Observations of Turtle Resting Sites in Kaneohe Bay, Oahu.

Reef	Site I.D.	Turtle I.D.	N	Sex	Duration (x $\pm$ s.d.) in seconds
42	A	20	1	Male	420
42	A	42	8	Male	329 $\pm$ 59
42	A	99	7	Male	369 $\pm$ 75
42	B	98	1	Undetermined	751
Mark's	C	97	1	Undetermined	694

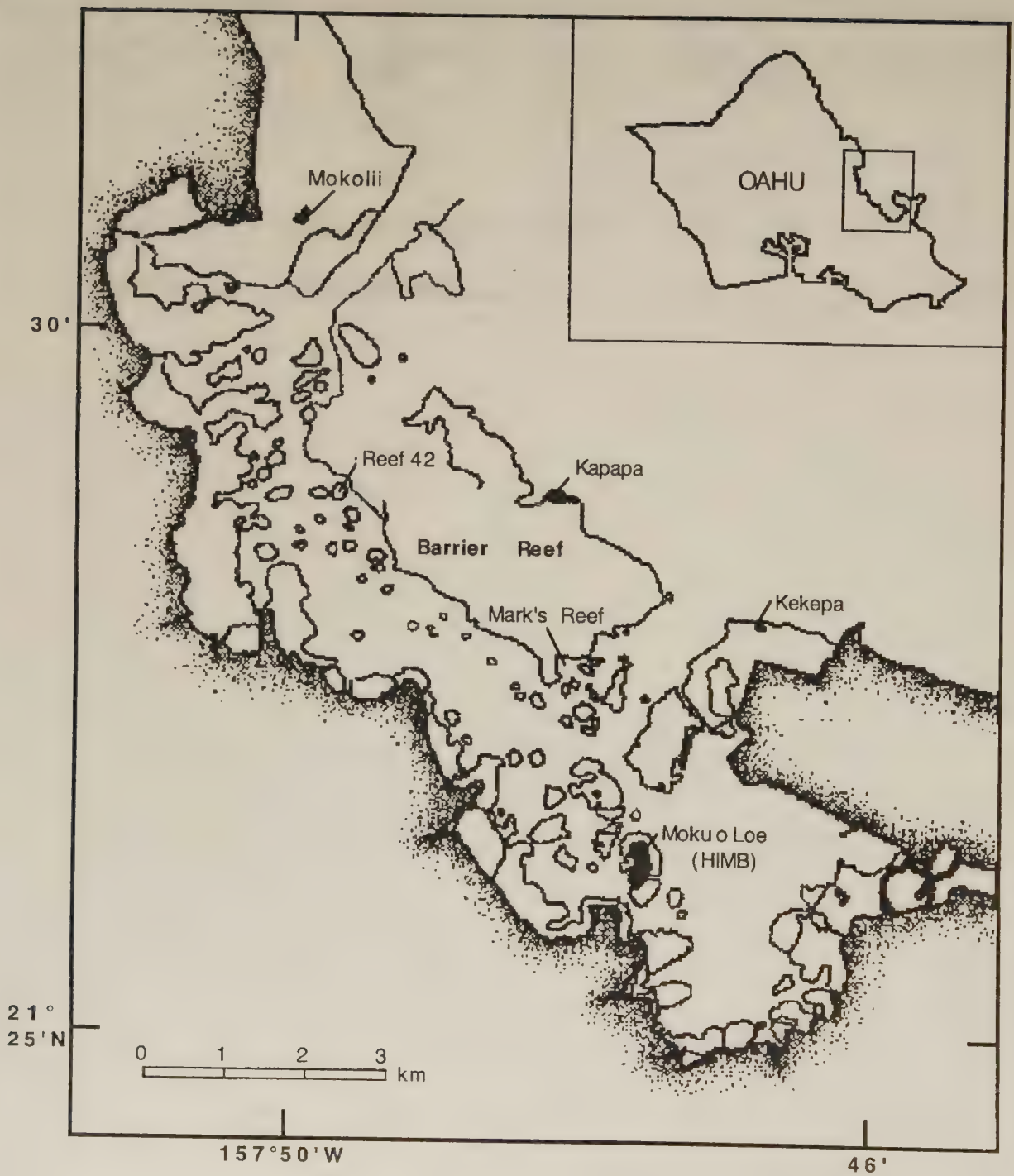


Figure 1.--Location of green turtle cleaning stations, Kaneohe Bay, Oahu, Hawaii.



Figure 2.--Location of cleaning station at Reef 42, Kaneohe Bay, Oahu, Hawaii ($21^{\circ}28.593'\text{N}$, $157^{\circ}49.451'\text{W}$).

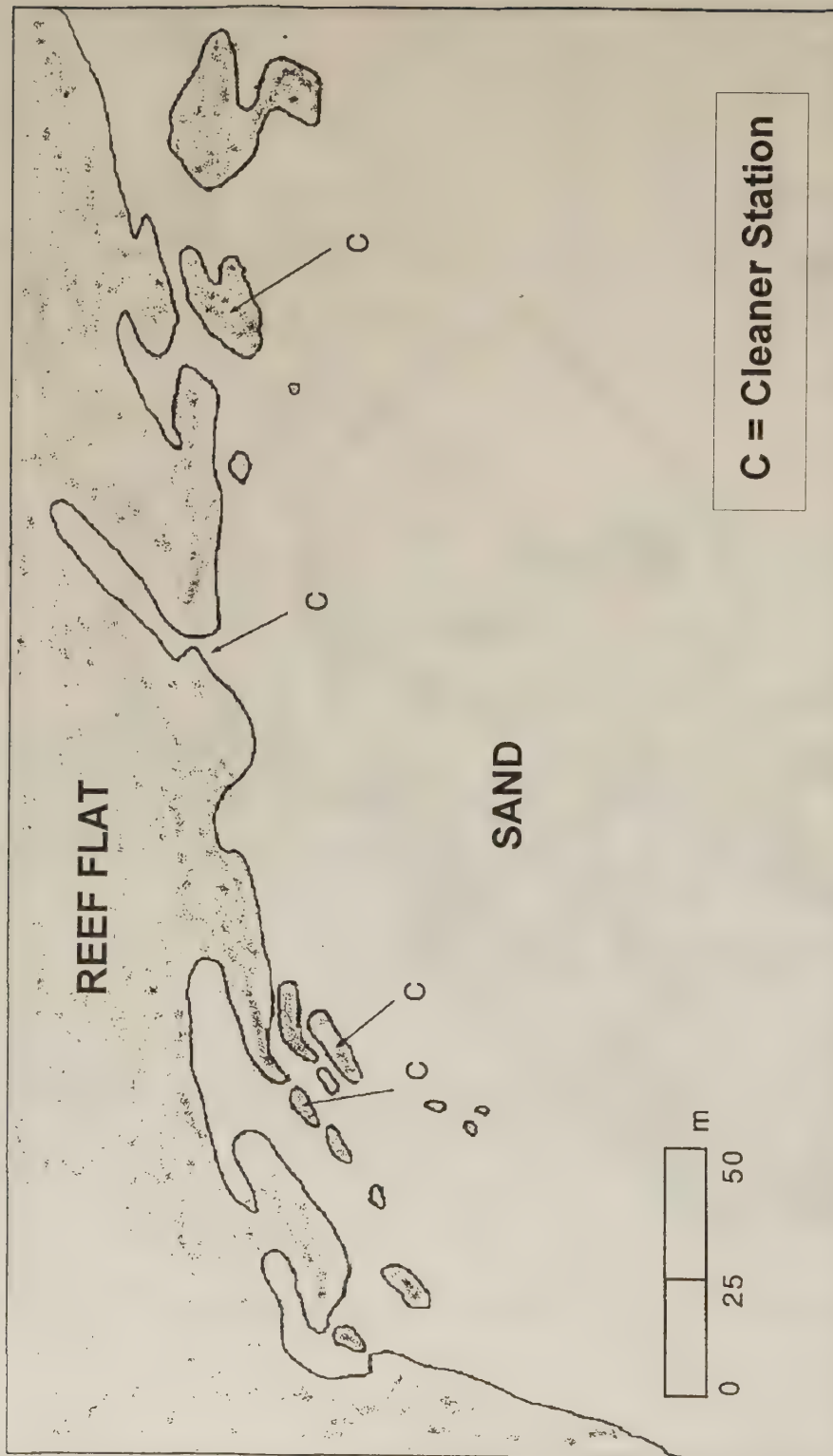


Figure 3.--Location of cleaning stations at Mark's Reef, Kaneohe Bay, Oahu, Hawaii
($21^{\circ}27.486\text{N}$, $157^{\circ}47.867\text{W}$).

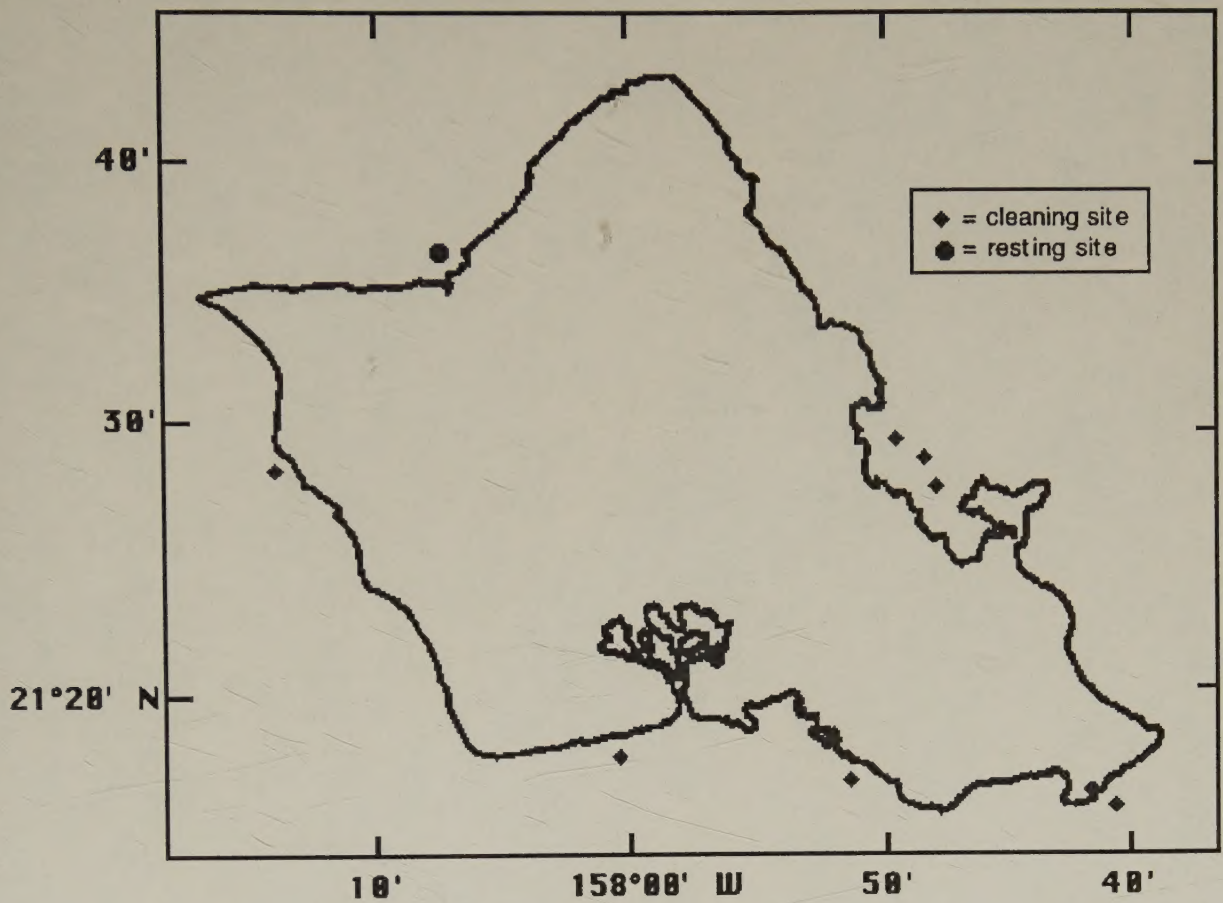


Figure 4.--Location of green turtle cleaning and resting stations around Oahu, Hawaii.

